CHAPTER 14

Rh and LW blood group antigens

Connie M. Westhoff1,2 & Don L. Siegel2
1Laboratory of Immunohematology and Genomics, New York Blood Center, New York, NY, USA
2Division of Transfusion Medicine and Therapeutic Pathology, Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Summary
Although the Rh and LW antigens are carried on entirely different proteins, they are incorporated together in this chapter on the basis of a historic serologic connection (and confusion) and evidence that they are physically associated within the red cell membrane.

Rh blood group system

History and nomenclature
The Rh system is second only to the ABO system in importance in transfusion medicine because Rh antigens, especially D, are highly immunogenic and cause hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs). HDFN was first described by a French midwife in 1609 in a set of twins, one of whom was hydropic and stillborn, whereas the other was jaundiced and died of kernicterus. That a wide range of observed clinical scenarios involving red cell hemolysis were related—from severely hydropic stillborn fetuses to infants with mild or significant levels of jaundice and kernicterus—was not realized until 1932. The cause of red cell hemolysis remained elusive until 1939, when Levine and Stetson described a woman who delivered a stillborn fetus and also suffered a severe hemolytic reaction when transfused with blood from her husband. Levine and Stetson correctly surmised that the mother had been immunized by a fetal red cell antigen inherited from the father and suggested that the cause of the erythroblastosis fetalis was maternal antibody in the fetal circulation. They did not give the target blood group antigen a name. Meanwhile Landsteiner and Wiener, in an effort to discover new blood groups, injected rabbits and guinea pigs with rhesus monkey red cells. The antisera they obtained agglutinated not only rhesus monkey red cells but also the red cells of 85% of White subjects, whom they called “Rh positive”; the remaining 15% of individuals studied were termed “Rh negative.” The “anti-Rhesus” serum seemed to be reacting similarly to the maternal antibody in serologic testing, hence the blood group system responsible for HDFN came to be known as “Rh.” The anti-Rhesus serum, in actual fact, was detecting the LW antigens (subsequently named for Landsteiner and Wiener), which is present in greater amounts on D-positive than on D-negative red cells.

The major Rh antigens are D, C, c, E, and e, but the Rh blood group system is one of the most complex because of the number of additional antigens that have been reported (Table 14.2). These additional antigens include compound antigens in cis (e.g., f [ce], Cw, cE, and CE), low-incidence antigens arising from partial-D hybrid proteins (e.g., D”, Go”, and Evans), and antigens arising from various point mutations in the RhCE protein (e.g., Cw’, Cw”, and VS). Table 14.2 also includes the numeric designations for Rh antigens. With a few exceptions (RH17 and RH29), the numeric designations are not widely used in the clinical laboratory.


176
### Genes and their expressed proteins

Two genes designated *RHD* and *RHCE* encode the Rh proteins. They are 97% identical, each has 10 exons, and they are the result of a gene duplication on chromosome 1p34–36. Rh-positive individuals have both genes, whereas most Rh-negative individuals have only the *RHCE* gene (see below).

RhD and RhCE are 417-amino-acid, nonglycosylated proteins. One protein carries the D antigen, and the other carries various combinations of the CE antigens (ce, cE, Ce, or CE). RhD differs from RhCE by 32–35 amino acids, depending on which form of RhCE is present (Figure 14.1). This relatively large degree of difference explains why D is the most immunogenic of all the blood group proteins, because most other blood group antigen polymorphisms result from only single amino acid changes in the respective protein. The Rh proteins migrate in sodium dodecyl sulfate polyacrylamide gels with an approximate molecular weight of 125 kDa, and hence were sometimes referred to as the 125 kDa proteins. They are predicted to span the membrane 12 times and are covalently linked to fatty acids (palmitate) in the lipid bilayer (Figure 14.1).

### Basis for antigen expression

**D antigen**

Rh-positive and Rh-negative refer to the presence or absence of the D antigen, respectively. The Rh-negative phenotype occurs in 15–17% of Whites, but is not as common in other ethnic populations and is very rare in Asia. The absence of D on the red cells of people of European ancestry was caused by a complete deletion of the *RHD* gene and occurred on a Dce (R_{Dc}) haplotype because the allele most often carried with the deletion is RHCE^ce. Deletion of the *RHD* gene is associated with being "Rh-negative" in all populations, but inactive or silenced RHCE is also a cause of D-negative phenotypes in Asians or Africans. D-negative phenotypes in Asians occur with a frequency of <1%, and carry mutations in *RHD* genes associated with RHCE^ce, indicating that they probably originated on a Dce (R_{Dc}) haplotype. Only 3–7% of South African blacks are D-negative, but 66% have *RHD* genes that contain a 37-bp internal duplication, which results in a premature stop codon. Additionally, 15% of D-negative phenotypes in Africans result from a hybrid *RHD* IIIa-CE-D gene that does not encode D epitopes. This is important when designing polymerase chain reaction (PCR)-based methods to predict the D status of the fetus and the possibility of HDFN. The population being tested, and the different molecular events responsible for D-negative phenotypes (i.e., gene deletion or gene mutation) must be considered. Even among D-negative whites, cases of a *RHD* gene that is not expressed because of mutation or nucleotide insertions have been reported.

### Table 14.1 Nomenclature and prevalence of Rh haplotypes

<table>
<thead>
<tr>
<th>Haplotype-Based Antigens (Fisher-Race)</th>
<th>Shorthand for Haplotype (Modified Wiener)</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whites</td>
</tr>
<tr>
<td>Dce</td>
<td>R_{1}</td>
<td>42</td>
</tr>
<tr>
<td>DcE</td>
<td>R_{2}</td>
<td>14</td>
</tr>
<tr>
<td>Dce</td>
<td>R_{0}</td>
<td>4</td>
</tr>
<tr>
<td>DCE</td>
<td>R_{x}</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ce</td>
<td>r</td>
<td>37</td>
</tr>
<tr>
<td>Ce</td>
<td>r'</td>
<td>2</td>
</tr>
<tr>
<td>cE</td>
<td>r'</td>
<td>1</td>
</tr>
<tr>
<td>CE</td>
<td>r''</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

### Table 14.2 International society for blood transfusion (ISBT) numerical terminology and symbols for Rh antigens

<table>
<thead>
<tr>
<th>Numeric</th>
<th>Symbol</th>
<th>Numeric</th>
<th>Symbol</th>
<th>Numeric</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH1</td>
<td>D</td>
<td>RH12</td>
<td>cE</td>
<td>RH48</td>
<td>JAL</td>
</tr>
<tr>
<td>RH2</td>
<td>C</td>
<td>RH18</td>
<td>h\textsuperscript{1}</td>
<td>RH49</td>
<td>STEM</td>
</tr>
<tr>
<td>RH3</td>
<td>E</td>
<td>RH29</td>
<td>&quot;total&quot;</td>
<td>RH50</td>
<td>FPPT</td>
</tr>
<tr>
<td>RH4</td>
<td>c</td>
<td>RH30</td>
<td>Go\textsuperscript{a}</td>
<td>RH51</td>
<td>MAR</td>
</tr>
<tr>
<td>RH5</td>
<td>a</td>
<td>RH31</td>
<td>h\textsuperscript{a}</td>
<td>RH52</td>
<td>BARC</td>
</tr>
<tr>
<td>RH6</td>
<td>ce or f</td>
<td>RH32</td>
<td>Rh32\textsuperscript{1}</td>
<td>RH53</td>
<td>IAHK</td>
</tr>
<tr>
<td>RH7</td>
<td>Ce</td>
<td>RH33</td>
<td>Rh33\textsuperscript{1}</td>
<td>RH54</td>
<td>DAK</td>
</tr>
<tr>
<td>RH8</td>
<td>C\textsuperscript{e}</td>
<td>RH34</td>
<td>H\textsuperscript{4}</td>
<td>RH55</td>
<td>LOCR</td>
</tr>
<tr>
<td>RH9</td>
<td>C\textsuperscript{e}</td>
<td>RH35</td>
<td>Rh35\textsuperscript{1}</td>
<td>RH56</td>
<td>CENR</td>
</tr>
<tr>
<td>RH10</td>
<td>V</td>
<td>RH36</td>
<td>Be\textsuperscript{a}</td>
<td>RH57</td>
<td>CEST</td>
</tr>
<tr>
<td>RH11</td>
<td>E\textsuperscript{a}</td>
<td>RH37</td>
<td>Evans</td>
<td>RH58</td>
<td>CELO</td>
</tr>
<tr>
<td>RH12</td>
<td>G</td>
<td>RH39</td>
<td>C-like</td>
<td>RH59</td>
<td>CEAG</td>
</tr>
<tr>
<td>RH17</td>
<td>H_{r}</td>
<td>RH40</td>
<td>Tar</td>
<td>RH60</td>
<td>PARG</td>
</tr>
<tr>
<td>RH18</td>
<td>Hr\textsuperscript{a}</td>
<td>RH41</td>
<td>Celike</td>
<td>RH61</td>
<td>CEVF</td>
</tr>
<tr>
<td>RH19</td>
<td>h\textsuperscript{a}</td>
<td>RH42</td>
<td>Ce\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH20</td>
<td>VS</td>
<td>RH43</td>
<td>Crawford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH21</td>
<td>C\textsuperscript{G}</td>
<td>RH44</td>
<td>Nou</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH22</td>
<td>CE</td>
<td>RH45</td>
<td>Riv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH23</td>
<td>D\textsuperscript{a}</td>
<td>RH46</td>
<td>Sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH26</td>
<td>c-like</td>
<td>RH47</td>
<td>Day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Weak D**

An estimated 0.2–1% of whites (and a greater percentage of blacks) have reduced expression of the D antigen, which is characterized by weaker than expected reactivity with anti-D typing reagents or, alternatively, as failure of such red cells to agglutinate directly with anti-D reagents, requiring the use of an indirect antiglobulin test for detection. The basis of weak expression of D is heterogeneous, but is primarily associated with the presence of point mutations in *RHD*. The mutations encode amino acid changes most often predicted to be intracellular or in the transmembrane regions of RhD rather than on the outer surface of the red cell (Figure 14.2). These mutations primarily affect the efficiency of insertion and, therefore, the quantity of protein in the membrane, and they may not affect the expression of D epitopes. This explains why most individuals with a weak-D phenotype can safely receive D-positive blood and do not make anti-D. There are over 80 different mutations known to cause weak-D expression (Type 1 through Type 84). The long history of transfusing patients who have weak-D red cells with D-positive blood suggests that weak-D Types 1, 2, and 3 (which represent the majority of whites with weak D) are unlikely to make anti-D. Predicting which mutations alter D epitopes based on structural analysis can be helpful, but documenting the RHD genotype of patients whose red blood cells (RBCs) type as D-positive but who make anti-D following stimulation with D-positive RBCs is definitive evidence of which mutations are associated with changes in D epitopes.

It is important that donor center typing procedures detect and label weak-D RBC units as D-positive, because they can stimulate the formation of anti-D in D-negative recipients. However, testing for weak expression of D antigen by indirect antiglobulin testing (IAT) is not required for transfusion recipients, who would then receive D-negative units without untoward effects. When weaker than expected reactivity with anti-D is seen when typing females of child bearing potential, *RHD* genotyping should be considered to guide transfusion therapy to conserve D-negative donor units,
always in short supply, and to determine candidates for Rh immune globulin. Patients with weak-D types 1, 2, and 3 are not at risk for anti-D and can safely receive D-negative blood and females are not candidates for Rh immune globulin.

A very weak form of D expression (Del), which cannot be detected by routine serology methods but can be demonstrated by adsorbing and eluting anti-D, is relatively common in Asians (10–30% of apparent D-negative). Red cells with very low levels of D are primarily of concern for donor testing, because they have stimulated anti-D in D-negative recipients.

**Partial D**
The D antigen has long been described as a “mosaic” because of the observation that some Rh-positive individuals produce anti-D when exposed to D antigen. It was hypothesized that the red cells of these individuals lack some part of D and that they can produce antibodies to the missing portion. Molecular genetic analysis has shown that the missing portions of RHD are replaced by corresponding portions of RHCE in the great majority of cases (Figure 14.3). The novel sequences of amino acids and the conformational changes that result from segments of RhD joined to segments of RhCE can generate new antigens (e.g., BARC, D\(^{30}\), FPTT, DAK, Go\(^{6}\), Evans, and Rh32) (Figure 14.3 and Table 14.2). The replacements are the result of gene conversion, the hallmark being that the donor gene is unchanged. Gene conversions involve single or multiple exons, whereas others involve short stretches of amino acids (Figure 14.3). Some also result from only single-amino-acid changes (e.g., DMH, DFW, and DII) (Figure 14.2). In contrast to the single-amino-acid changes that cause weak D (above), which are predicted to be cytoplasmic or transmembrane in location, those that cause partial-D phenotypes are often predicted to be located on the extracellular loops of the protein (Figure 14.2).

From a clinical standpoint, individuals with partial-D antigens are at risk for anti-D, and ideally females of childbearing potential with partial D should receive D-negative blood and, if they become pregnant, are candidates for Rh immune globulin. Some RBCs with partial D react weaker than expected with anti-D reagents, but they cannot be distinguished from weak-D RBCs, which are not at risk for anti-D. RHD genotyping can guide clinical decision making for Rh immune globulin prophylaxis and transfusion. In practice, many individuals with partial-D phenotypes will type strongly D-positive and will be recognized only after they have made anti-D following a transfusion with D-positive cells or pregnancy with a D-positive fetus. Routine RHD genotyping would overcome the limitations of serologic D typing of these individuals.

**Elevated D**
Several rare phenotypes, including D\(^{30}\), Dc\(^{20}\), and DCw\(^{30}\), have enhanced expression of D antigen and no, weak, or variant CE antigens, respectively. These phenotypes are analogous to the partial-D rearrangements described in the “Partial D” subsection, only they involve the opposite situation—that is, replacement of portions of RHCE by RHD. The additional RHD sequences in RHCE along with a normal RHD explain the enhanced D and account for the reduced or missing CE antigens. Although these represent altered RHCE genes (see below), they are included here because of their elevated D phenotype. Individuals with such altered CE phenotypes can make anti-Rh17 when immunized. Anti-Rh17 reacts with all cells that have conventional Rhce, RhCe, or RhcE protein, and only RBCs with the D\(^{30}\) phenotype (or those expressing the same Rhce variant) are compatible.

**C/c and E/e antigens**
The four major forms of the RHCE gene encode four different proteins: RhCe, cE, eE, and CE (Figure 14.1). C and c differ by four amino acids: Cys16Tyr encoded by exon 1, and Ile60Leu, Ser68Asn, and Ser103Pro encoded by exon 2 (Figure 14.1, open circles on RhCe). Of those four amino acids, only the residue at 103 is predicted to be extracellular and is located on the second loop. All the amino acids encoded by exon 2 of RHCE Ce are identical to those encoded by exon 2 of RHD (Figure 14.1, dark blue on RhCe). This suggests that RHCE Ce arose from the transfer of exon 2 from RHD into an RHCE ce gene, respectively. The sharing of exon 2 encoded amino acids by RhD, RhCe, and RhCE accounts for the expression of the G antigen on red cells that are D or C positive.
Individuals who lack D and C can make anti-G, which can be distinguished from a combination of anti-D and anti-C by absorption and elution studies.\(^5\) The presence of anti-G can explain why a D-negative person who was transfused with D-negative/C-positive red cells (or exposed to such cells through pregnancy) can subsequently appear to have made anti-D. The identification of anti-G is generally of academic interest only because transfusion requirements for such an individual would be the same (i.e., RBCs that are both D and C negative). However, in the case of a pregnancy, it is important to identify individuals with anti-G only, as Rh immune globulin prophylaxis would be indicated.

E and e differ by one amino acid, Pro226Ala, predicted to reside on the fourth extracellular loop of the protein (Figure 14.1, solid circle). The E antigen arose from a single point mutation that occurred in exon 5 of the \(RHCE^*\) gene, giving rise to \(RHCE^*cE\). \(^{22}\)

V and VS antigens, which are expressed on RBCs of more than 30% of blacks, result from a Leu245Val substitution located in the predicted eighth transmembrane segment of Rhce (Figure 14.1).\(^{23}\) The V–VS+ phenotype results from a Gly336Cys change on the 245Val background.\(^{24}\) V+ and VS+ are associated with weak and altered expression of e, indicating that Leu245Val causes a local conformation change on the fourth extracellular loop where the e-specific amino acid resides.

Most individuals have e-positive red cells, but the e antigen is considered to be second in complexity to D because variant expression has frequently been observed.\(^{25}\) The e antigen is altered on many red cells from African blacks, and some of the alleles encoding altered expression are shown (Figure 14.3). Extensive discussion is beyond the scope of this chapter, but these alleles are prevalent in patients with sickle cell disease (SCD),\(^{26,27}\) who not infrequently produce anti-e following transfusion, despite having an e-positive red cell phenotype. RH genotyping of the patient can help to determine if the anti-e is allo- or auto-antibody and to inform selection of blood for transfusion.\(^{28}\)

E variants are not common and include EI, EII, and EIII, which result from a point mutation (EI) or gene conversion replacement of Rhce amino acids with RhD residues (EII and EIII) with concurrent loss of some E epitope expression. Category EIV red cells, which

---

**Figure 14.2** Predicted location of point mutations that cause weak-D and partial-D phenotypes. Weak-D phenotypes carry mutations in \(RHD\) that primarily cause amino acid changes predicted to be intracellular or in the transmembrane regions of D (upper panel). The specific amino acid mutation and the location in the protein are indicated. The partial-D phenotypes that carry mutations in \(RHD\) are predicted to be located on extracellular loops of RhD (lower panel). The specific amino acid mutation and location in the protein, as well as the common name for each, are indicated.

**Altered CE**

C\(^w\) and C\(^a\) are low-incidence antigens that result from single-amino-acid changes (Gln41Arg and Ala36Thr, respectively) predicted to be located on the first extracellular loop of RhCE (Figure 14.1, light blue circles).\(^{22}\) These antigens are more common in Finns (4%) and are most often present on RhCe. C\(^w\) is also associated with the deletion phenotype DC\(^w\)– (Figure 14.3).
have an amino acid substitution in an intracellular domain, do not lack E epitopes but have reduced E expression.\textsuperscript{28}

Variants of c are infrequent. The very rare RH:-26 results from a Gly96Ser transmembrane amino acid change that abolishes Rh26 and weakens c expression.\textsuperscript{29} The lack of c antigen variants in humans compared to the other Rh antigens, and the preservation of expression of c on the red cells of nonhuman primates, suggest that the two proline residues involved form a stable structure that is resistant to perturbations and changes in Rhce.\textsuperscript{30}

In summary, point mutations and genetic exchange, mainly involving gene conversion events between \textit{RHD} and \textit{RHCE}, are primarily responsible for the large number of Rh antigens. Additional complexity results because many of the Rh epitopes are highly conformational, and single-amino-acid changes in one part of the protein, including changes within the transmembrane regions, can affect the expression of cell-surface-exposed antigen epitopes.

**RH genotyping**

DNA-based testing methods were introduced to the blood bank and transfusion medicine community over two decades ago. Assays for blood group antigens encoded by single nucleotide polymorphisms (SNPs) are highly reproducible and correlate with red cell phenotype. Semi-automated testing platforms are now being used for routine antigen typing.\textsuperscript{31} Genotyping for the two most important blood group systems, ABO and Rh, are more challenging because of the many different mutations that cause weak expression of A and B, and inactive O, and because of the numerous variant Rh antigens and hybrid RH alleles. Multiple regions of the genes must be sampled, and hybrid genes are problematic for analysis. RH genotyping is performed principally in a reference laboratory setting at the present time.

**RHD zygosity testing**

Serologic testing for red cell expression of D, C/c, and E/e can only predict the likelihood that a sample is homozygous (D/D) or heterozygous (D/\textit{D\textasciitilde}) for \textit{RHD}. Genotyping enables zygosity to be determined by assaying for the presence of a recessive D-negative allele. In prenatal practice, paternal \textit{RHD} zygosity testing is important to predict the fetal D status when the mother has anti-D. Several different genetic events cause a D-negative phenotype, and multiple assays must be performed to accurately determine zygosity. If the father is \textit{RHD} homozygous, the fetus will be D-positive, and monitoring of the pregnancy will be required. If the father is heterozygous, the D type of the fetus can then be determined to prevent invasive and unnecessary testing.

**Fetal typing**

Genotyping is important in the prenatal setting to determine whether the fetus has inherited the paternal antigen to which the mother has a clinically significant antibody. Fetal DNA can be isolated from cells obtained by amniocentesis. The discovery that cell-free, fetal-derived DNA is present in maternal plasma or serum...
by approximately five weeks gestation allows maternal plasma to be used as a source of fetal DNA. The small quantity of cell-free fetal DNA present relative to maternal DNA requires positive controls for isolation of sufficient fetal DNA to validate negative results. Isolation of fetal DNA from maternal plasma has become routine in several European countries to determine fetal D status prior to administration of Rh immune globulin. This has been most successful for D typing because the D-negative phenotype in the majority of samples is caused by the lack of RH. Testing for the presence or absence of a gene is less demanding than testing for a single gene polymorphism or SNP. Testing the maternal plasma for the presence of a fetal RHD eliminates the unnecessary administration of antepartum Rh immune globulin (RhIG) to the approximately 40% of D-negative women who are carrying a D-negative fetus. RhIG is not entirely risk free as it is a human blood product, and this approach is cost-effective for some healthcare systems. Noninvasive testing for fetal K and HPA-1a status has also been reported.

**Distinction between weak D and partial D**

As indicated above, altered expression of D antigen is not uncommon. Weak-D phenotypes have amino acid changes that primarily affect the quantity of RhD in the membrane. Partial-D phenotypes have amino acid changes that alter D epitopes, or often are hybrid proteins with portions of RhD joined to portions of RhCE. The distinction between weak-D and partial-D phenotypes is of clinical importance because the latter make anti-D. Routine serologic typing reagents cannot distinguish between these red cells; however, RHD genotyping strategies can discriminate between weak D and partial D.

**Detecting patients at risk for production of antibodies to high-incidence antigens**

alloimmunization is a serious complication of chronic transfusion, particularly in patients with SCD requiring long-term transfusion support. Many transfusion programs attempt to prevent or reduce the risk and incidence of alloantibody production in patients with SCD by transfusing RBC units that are antigen matched for D, C, E, and K. Although this approach reduces the incidence of alloimmunization, variant RHD and RHCEece genes are common in African blacks and individuals of mixed ethnic backgrounds. The prevalence of RH alleles that encode altered D, C, and E antigens in this patient group explains why some SCD patients become immunized to Rh, despite Rh antigen matching for D, C, and E. These antibodies often have complex, high-incidence Rh specificities, and it can be difficult or impossible to find compatible units. RH genotyping can identify those patients who are homozygous for variant RH alleles and at risk for production of alloantibodies to high-incidence Rh antigens, and can identify compatible donors for transfusion.

**Rhnull phenotype**

Rhnull individuals lack expression of all Rh antigens. They suffer from a compensated hemolytic anemia, and they have variable degrees of spherocytosis, stomatocytosis, and increased red cell osmotic fragility. The phenotype is rare and occurs on two different genetic backgrounds: the “regulator” type, caused by mutation in the RHAG gene at an unlinked locus, and the “amorph” type, which maps to the RH locus. In the more common regulator type of Rhnull, the suppression of Rh antigens is caused by a lack of, or a mutant, Rh-associated glycoprotein (RhAG) protein. RhAG is a 409-amino-acid glycosylated protein that co-immunoprecipitates with RhD and RhCE. It shares 37% amino acid identity with the RhD-RhCE proteins and has the same predicted membrane topology. RhAG is not as polymorphic as RhCE and RhD, and only four antigens have been identified; however, it is important for targeting the Rh proteins to the membrane during erythroid maturation. RhAG has one N-glycan chain that carries ABO and Ii specificities.

The amorph type of Rhnull results from mutations in RHCE on a D-negative background. Amorph-type red cells express no Rh protein and have reduced amounts (~20%) of RhAG.

**Rh membrane complex**

Additional complication in Rh protein structures arises because they exist in the red cell membrane as complexes with several other proteins. Rh and RhAG are associated in the membrane as a core complex. The fact that several other proteins interact with the Rh core complex is based on observations of Rhnull cells. These red cells have reduced expression of CD47, an integrin-associated protein (IAP) that has wide tissue distribution, binds β3 integrins, and is required for integrin-regulated Ca2+ entry into endothelial cells. Its function on the red cells is unknown; however, a role in red cell senescence has been suggested. Rhnull cells also have reduced glycoporphin B (GPB), a sialoglycoprotein that carries S or s and U antigens. GPB appears to aid RhAG trafficking to the membrane, because the RhAG protein in GPB-deficient cells has increased glycosylation, reflecting longer dwell time in the endoplasmic reticulum. Rhnull cells also lack LW, a glycoprotein of unknown function that belongs to the family of intercellular adhesion molecules (ICAM-4). Band 3 (the anion exchanger) enhances the expression of the Rh antigens in transfected cells, suggesting that band 3 may also be associated with the Rh core complex. The Rh core complex is linked to the membrane skeleton through interactions between CD47 and protein 4.2 and through a novel Rh/RhAG–ankyrin cytoskeleton connection.

**Rh function**

**Rh glycoproteins (RhAG, RhBG, and RhCG)**

The Rh blood group proteins are well known because of their importance in blood transfusion. However, the mammalian family of Rh proteins also includes RhAG in erythrocytes, and the related proteins, RhBG and RhCG, in other tissues.

Protein sequences with similarities to the mammalian Rh proteins were first found in *Caenorhabditis elegans*, and these homologs, in turn, showed similarity to the ammonia transporters from bacteria, yeast (MEP), and plants (AMT). The relationship of the Rh glycoproteins to the AMT and MEP ammonia transporters from these other organisms has been substantiated by functional transport data and structural modeling. The Rh proteins reveal the power of comparative genomics and proteomics, in which sequence analysis and homology modeling can give important insight into mammalian protein function.

The non-erythroid Rh glycoproteins, RhBG and RhCG, are present in the kidney, liver, brain, and skin where ammonia production and elimination occur. In the kidney collecting segment and collecting duct, RhBG and RhCG are found on the basolateral and apical membranes, respectively, of the intercalated cells where they mediate transepithelial movement of ammonia from the interstitium to the lumen. In the liver, RhBG is found on the...
basolateral membrane of perivenous hepatocytes, where it may function in ammonia uptake. RhCG is also present in bile duct epithelial cells, where it is positioned to contribute to ammonia secretion into the bile fluid.56

The mechanism of ammonia transport by Rh glycoproteins is an electroneutral process that is driven by the NH4+ concentration and the transmembrane H+ gradient.57,58 Functional studies of the kidney, liver, and brain Rh homologs, along with the erythrocyte RhAG/Rh proteins, promise to lead to development of a unifying hypothesis of ammonia transport in mammals by the Rh family of proteins.

RhCE and RhD
The more recently evolved erythrocyte blood group proteins, RhCE and RhD, when expressed in heterologous systems do not transport ammonia.59 RhCE and RhD lack the highly conserved histidine residues located in the membrane pore that are critical for ammonia transport. RhCE and RhD may be evolving a new function in the red cell membrane as phylogenetic analysis indicates the RhCE/D proteins are rapidly evolving.

Clinical relevance of Rh protein expression in RBCs
Lack of Rh expression (i.e., Rh-null RBCs) most often results from absence or mutations in RhAG. RhAG is important for ion balance in RBCs.60,61 Mutations in the RhAG ammonia conductance channel are associated with altered ion transport. Hereditary overhydrated stomatocytosis (OHSt), an autosomal dominant macrocytic hemolytic anemia, is caused by a Phe65Ser mutation in RhAG. OHSt red cells exhibit cation leak with elevated Na+ and reduced K+ content, and loss of ammonia conductance.

Immune response to Rh
Medical aspects
Human red cells can express more than 400 different blood group antigens. Typing patient and donor cells for every known antigen with the intention of providing perfectly matched blood would not be practical or feasible. Fortunately, such extensive testing is not required for a number of reasons, the most important of which is that exposure to the majority of foreign red cell antigens through transfusion does not lead to the production of clinically significant alloantibodies. D is one notable exception. As many as 80% of D-negative patients on deliberate immunization with D-positive red cells may develop high-titer, high-affinity antibodies to D.62 D IgG antibodies that initiate complement activation, the vast majority of anti-Rh-containing sera do not do so. The usual explanation for this cites the relatively low copy number of Rh antigens per red cell, which results in Rh molecules situated too far apart on the cell surface to permit the simultaneous binding of C1q by multiple Rh IgG antibodies. Therefore, hemolysis from the transfusion of Rh-incompatible RBCs is generally extravascular because of the phagocytosis of IgG-coated erythrocytes by cells of the reticuloendothelial system.

In practice, D-positive patients can be transfused with either D-positive or D-negative RBCs—the absence of D will cause no harm—but it is deemed prudent to reserve the rarer units of D-negative blood (~15% of donor units) for D-negative individuals who must receive them. In cases of trauma and/or massive transfusion in which the patient’s D status is unknown, efforts are made to provide D-negative blood, especially for females of childbearing potential, until the appropriate testing can be completed. When D-negative blood is in short or critical supply, it may be necessary to transfuse D-negative patients with D-positive units. In such scenarios, D-negative units are reserved for females of childbearing potential and for patients whose serum contains anti-D from a previous sensitization.

Unlike ABO blood group antigens, which are expressed by all transfused blood cells including platelets, the D antigen is present only on red cells. Theoretically, the selection of platelet units for transfusion should be independent of the D status of the donor. However, a transfusion of pooled platelet concentrates may introduce as much as 5 mL of donor red cells, which may be sufficient to alloimmunize a D-negative patient. Therefore, the standard of care is to avoid transfusing D-negative patients, particularly females of childbearing potential, with platelet units derived from D-positive donors. If such units are unavailable and platelet transfusion must be undertaken, the administration of RhIG can be considered. A standard 300-μg dose of RhIG, which may inhibit the immunizing potential of up to 15 mL of D-positive red cells, would neutralize the effects of D-positive red cells from several mismatched platelet transfusions. If mismatched platelet transfusions are repeatedly given over time and their content of D-positive red cells is not expected to exceed the volume of red cells for which a standard dose of RhIG is indicated, then a single dose of the drug should be sufficient for at least 2–4 weeks of prophylaxis given the 3-week half-life of IgG.63 With respect to the transfusion of plasma products, the D status of the donor is not an issue because plasma products do not contain cellular or soluble material capable of inducing anti-D immune responses.

Serologic aspects
The immune response to Rh, like that to other peptide antigens, is typically thymus-dependent, requiring T-cell help. Upon exposure to a foreign Rh antigen, an IgM response may develop, but this is quickly followed by the production of IgG antibodies. Consequently, nearly all examples of Rh antibodies are IgG molecules (mostly IgG1 and IgG3), which bind optimally to red cells at 37°C and require the addition of an antihemoglobin reagent to produce hemagglutination. Although IgG1 and IgG3 subclasses classically initiate complement activation, the vast majority of anti-Rh-containing sera do not do so. The usual explanation for this cites the relatively low copy number of Rh antigens per red cell, which results in Rh molecules situated too far apart on the cell surface to permit the simultaneous binding of C1q by multiple Rh IgG antibodies. Therefore, hemolysis from the transfusion of Rh-incompatible RBCs is generally extravascular because of the phagocytosis of IgG-coated erythrocytes by cells of the reticuloendothelial system.

After anti-D, the Rh antibodies most commonly found in the sera of alloimmunized individuals are anti-E > anti-c > anti-e > anti-C. In approximately 50% of cases of warm-type autoimmune hemolytic anemia (WAIHA), autoantibodies are believed to be directed to Rh antigens by virtue of their “pan-reactivity” with all red cell phenotypes except Rhnull cells. However, direct binding of autoantibodies to putative epitopes common to D and C/E polypeptides or to other components of the Rh membrane complex (RhAG, CD47, etc.) has yet to be demonstrated in WAIHA. The difficulties in approaching this problem are largely technical in nature and relate to both the inability to produce workable quantities of pure
patient autoantibody in vitro (i.e., clone the autoantibody-producing B lymphocytes) and the inability to purify Rh proteins in a way that retains their native, conformationally dependent epitopes.

Molecular aspects
The characterization of Rh antibodies on a molecular level, particularly that of anti-D, has been the focus of much study not only because of their clinical significance, but also because of the need to develop suitable in vitro methods for their production.63 Ironically, because of better transfusion practice and the use of RhIG, alloimmunization of antigen-negative individuals is significantly less common (as are sera donors willing to be purposely hyperimmunized), so that supplies of Rh antibodies for use as typing reagents and for the preparation of RHig are dwindling. To better understand the molecular makeup of Rh antibodies, investigations have focused on analyzing their variable regions in order to determine whether there are commonly shared genetic and/or structural features among Rh antibodies made by different individuals.

Early work using rabbit antisera specific for different human heavy-chain variable region gene products suggested a restriction in the use of certain heavy-chain gene families by the anti-D contained in polyclonal sera from several dozen anti-D donors.64 Subsequent studies with rodent idiotype antibodies demonstrated cross-reactive idiotypes among polyclonal anti-D preparations65,66 and among different examples of human monoclonal anti-C, -c, -D, -E, -e, and -G produced by transformed B cells.67 A more direct approach using nucleotide sequencing to examine immunoglobulin gene diversity examined a cohort of four IgM and 10 IgG monoclonal anti-D variable regions.67 A restricted use of the human heavy-chain variable region genes $V_{H}$–33 and $V_{H}$–34 was found with a shift in repertoire usage toward $V_{H}$–33 for anti-D that had isotype switched to the more clinically relevant IgG. The restriction of anti-D heavy chains to the use of these and other highly related $V_{H}$ genes has been confirmed and extended through the analysis of many additional examples of anti-D produced through both tissue culture and recombinant means.68–73 The use of molecular approaches such as site-directed mutagenesis,74 complementarity-determining region (CDR) sequence randomization,75 and heavy-chain/light-chain ”shuffling”76 has demonstrated the genetic relatedness among anti-D molecules directed against different D epitopes as well as among antibodies with D and E specificity. These studies and others77,78 have supported the hypothesis that a restricted “Rh footprint” for D alloantibodies and a process termed “epitope migration”68 play a role in the molding of the anti-Rh immune repertoire.79

Although the precise significance of immunoglobulin germline gene restriction by Rh antibodies is not fully understood, it may have practical significance for the preparation of anti-D for both therapeutic and diagnostic use. For example, the $V_{H}$ genes used to encode anti-D are among the most cationic of the human germline $V_{H}$ genes80 and may account for the relatively high pI of polyclonal antibodies that make contact with the D antigen to the effector functionality of the anti-D, particularly the role that Fe glycosylation may play in inducing immune prophylaxis.63,85,86

**LW blood group system**

**History and nomenclature**
The LW antigens are the “true” Rhesus antigens shared by humans and the rhesus monkey. As discussed earlier, the confusion occurred because LW antigens are more abundant on D-positive than on D-negative red cells. When the situation was clarified, the term Rh remained associated with the human antigen, so the real Rhesus antigen was renamed LW in honor of Landsteiner and Wiener.5 The confusion can be understood today in the transfusion service when a weak example of anti-LW often appears initially to be anti-D. The LW system has undergone additional terminology revisions. The historical terminology of LW1, LW2, LW3, LW4, and LW0 to describe phenotypes was based on both the LW and the D status of the red cells but is now obsolete.5 The phenotypes are LW(a+b−), LW(a−b+), LW(a+b+), and the rare LW(a−b−); the antigens are designated LWα, LWβ, and LWab.

**Genes and their expressed proteins**
LW is encoded by a single gene located on chromosome 19. The 42-kD LW glycoprotein is a member of the family of ICAMs and has been renamed ICAM-4 (CD242). LW passes through the red cell membrane once, and the N-terminal extracellular region is organized into two immunoglobulin superfamily (IgSF) domains.57

**Basis for antigen expression**
LWα is the common antigen, whereas LWβ has an incidence of less than 1% in most Europeans.63 The LWα/LWβ polymorphism is caused by a single amino acid substitution, Gln70Arg, on the LW glycoprotein.68 An increased frequency of the uncommon LWβ antigen in Latvians and Lithuanians (6%), Estonians (4%), Finns (3%), and Poles (2%) suggests that the LWβ mutation originated in the people of the Baltic region. The LWab antigen was originally defined by an alloantibody made by the only known (genetically verified) LW(a−b−) person, who lacks expression of all LW antigens.5 Rh antigen expression was not altered, and there was no clinical evidence of an associated pathology. The LW gene in this rare LW(a−b−) individual has a 10-bp deletion and a premature stop codon in the first exon.80 Rhnull red cells also lack LW antigens but do not have defective LW genes. Rh proteins appear to be required for LW to traffic to the membrane, and association with RhD is preferred.

LW antigens require divalent cations (e.g., Mg2+) for expression and have intramolecular disulfide bonds that are sensitive to dithiothreitol (DTT) treatment.5 This is helpful to differentiate anti-LW from anti-D, because the D antigen is resistant to DTT. DTT-treated and untreated D-positive red cells can be prepared and tested for reaction with patient serum.90 Also helpful in identifying
anti-LW is the fact that LW antigens are expressed equally well on group O D-positive and D-negative cord blood red cells. Transient loss of LW antigens has been described in pregnancy and patients with diseases, particularly Hodgkin’s disease, lymphoma, leukemia, sarcoma, and other forms of malignancy, in the absence of any overt associated RBC abnormality. Transient loss of LW antigens is associated with the production of autoanti-LW that can appear to be alloantibody.

**LW function**

LW glycoprotein, ICAM-4, is a ligand with broad specificity for a number of β1, β2, β3, and β5 integrins, including α6β1 (LFA-1), α6β2 (Mac-1), α4β1 (VLA-4), α5β1, αvβ3, as well as platelet integrin αIIbβ3. In addition, LW binds to the I domains of CD11a/CD18 and CD11b/CD18 on leukocytes. The function of LW glycoprotein on mature red cells is not known, but LW may have a role in erythrophagocytic island formation through interaction with macrophage integrins. LW appears to play a pathophysiological role in the development of vaso-occlusion in SCD mediated by endothelial cell integrins and of thrombosis mediated by activated integrins on platelets.

**Summary**

The molecular basis for the Rh antigens has now been elucidated. A gene deletion or silent RHD gene explains the absence of the D antigen in many Rh-negative individuals. The large number of amino acid differences between the RhD and RhCE proteins explains why exposure in an individual lacking D often results in a vigorous immune response characterized by a very heterogeneous anti-Rh immune repertoire. Questions remain concerning the function of the Rh proteins. The discovery that Rh protein homologs also exist in the liver and kidney indicates that the Rh blood group antigens belong to a conserved family of proteins that function in ammonia transport. Similarly, the function of LW on the mature red cell is not entirely clear, but its ability to interact as an adhesion molecule with a broad range of integrin-binding specificity suggests an important role in both normal red cell development as well as disease-associated processes such as vaso-occlusion and thrombosis.

**Key references**

A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion